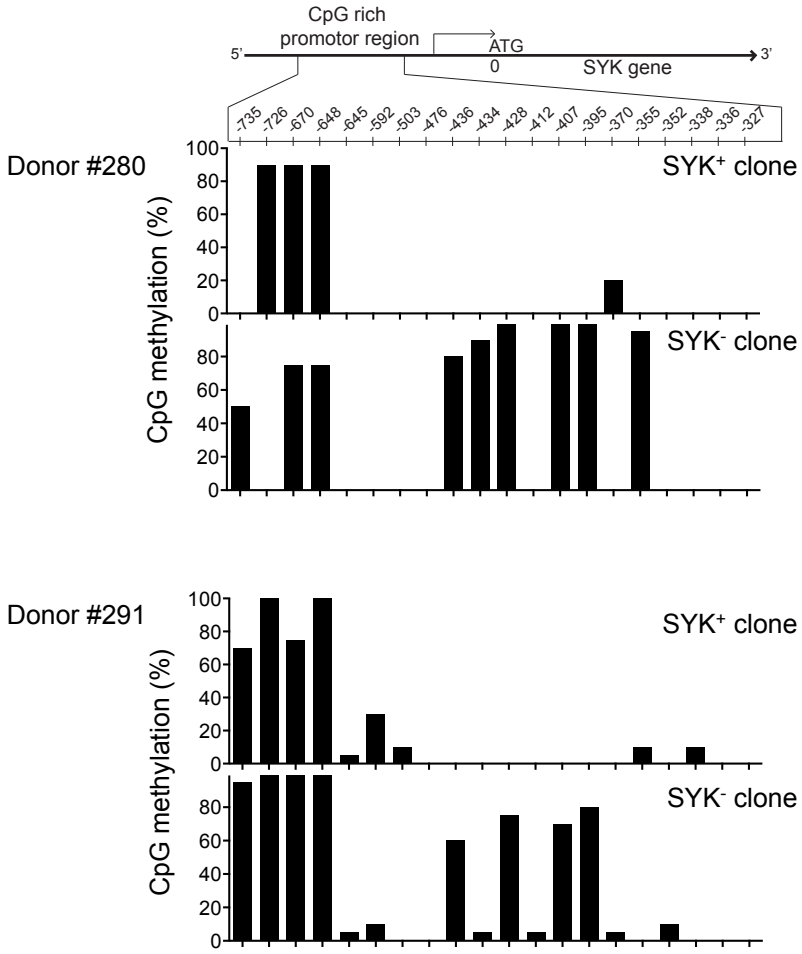

Figure S1 : Schematic diagram of the SYK gene including promoter-associated CpG island and translation start codon. Arrow represents the transcription initiation site. Expanded region details the location of 20 specific CpG dinucleotides as potential methylation sites. Bar graphs show percentage of methylation detected at each individual site in SYK-expressing and SYK-deficient NK cell clones from two donors.

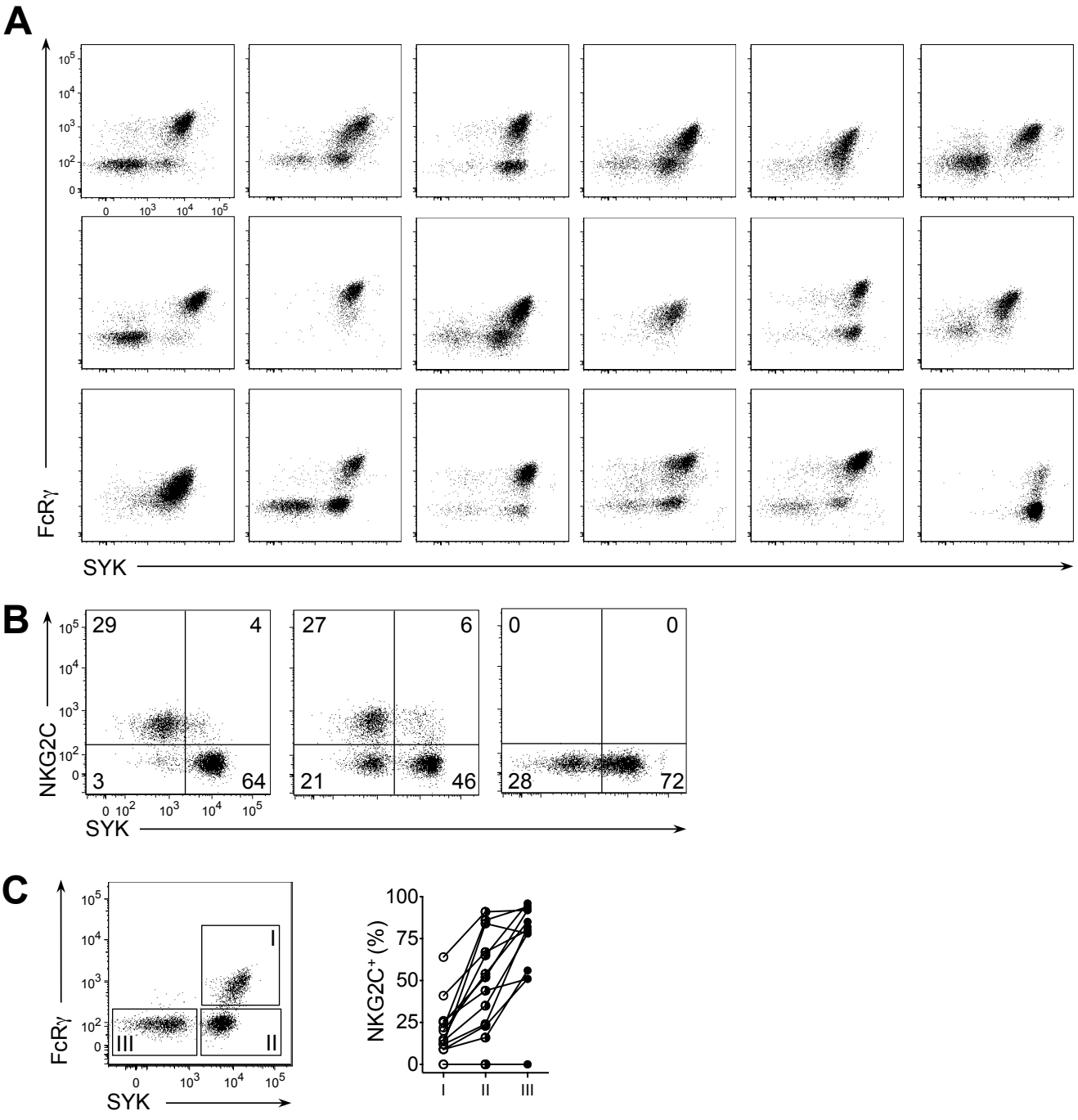

Figure S2 : Heterogeneous expression of FcRγ and NKG2C in SYK-deficient NK cells. (**A**) Dot plots show expression of SYK vs FcRγ in $CD56^{+}CD3^{-}CD19^{-}CD14^{-}$ NK cells from 18 HCMV seropositive donors. When SYK-deficient NK cells are present, they are largely confined within the $FcR\gamma^{-}$NK cell population in most donors. (**B**) Dot plots show expression of NKG2C vs. SYK in $CD56^{+}CD3^{-}CD19^{-}CD14^{-}$ NK cells from 3 representative HCMV seropositive donors. Numbers represent percentage of cells in each quadrant. (**C**) Dot plot shows 3 populations of NK cells designated as $FcR\gamma^{+}SYK^{+}$ (I), $FcR\gamma^{-}SYK^{+}$ (II), and $FcR\gamma^{-}SYK^{-}$ (III). Dot graphs show percentages of indicated NK cell subsets that express NKG2C in HCMV seropositive donors (*n*=14); two donors were completely negative for NKG2C expression. Circles connected by a line designate the same donor sample.

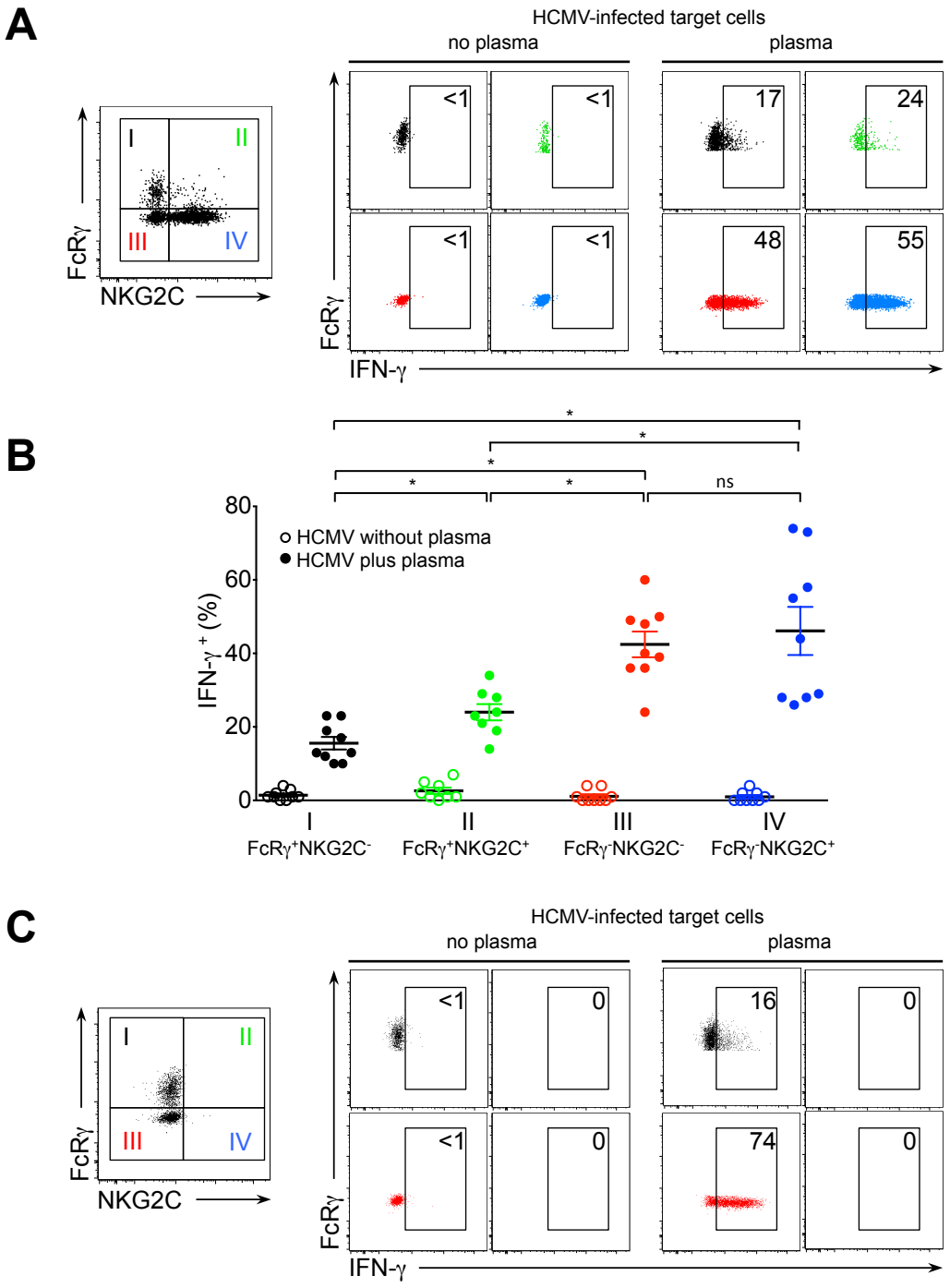

Figure S3 : Enhanced effector function by FcRγ$^-$NK cells in response to HCMV-infected target cells regardless of NKG2C expression. PBMCs were cultured with mock- or HCMV-infected MRC-5 cells as indicated. (**A**) Flow cytometric analysis of IFN-γ production by four distinct subsets (I, II, III, and IV) phenotypically segregated according to expression of FcRγ and/or NKG2C from a representative donor. Inset values represent the relative percentage of IFN-γ$^+$ NK cells. (**B**) Dot graph shows the percentages of specified subsets that produced IFN-γ$^+$ from several donors (*n*=9) that had at least 5% of the total NK cells in each of the four subsets in the absence (○) or presence (●) of autologous plasma as indicated. (**C**) Production of IFN-γ by conventional NK or FcRγ$^-$NK cells in the presence or absence of autologous plasma from a representative donor of two donors that do not express NKG2C at all. ns, not significant; * $P < 0.05$

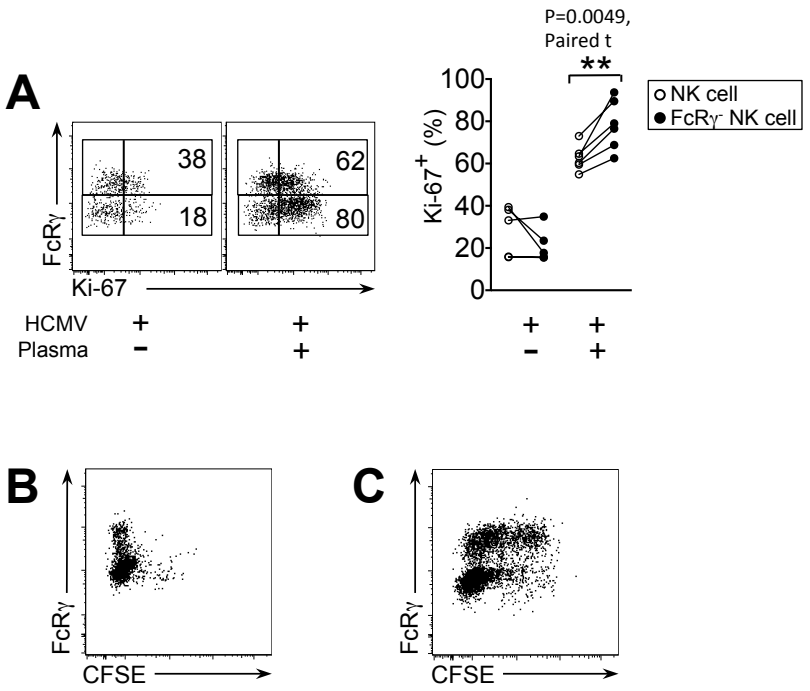

Figure S4: Preferential expansion of FcRγ$^-$NK population in response to HCMV-infected target cell and HCMV sero-positive plasma. (**A**) PBMCs were cultured in the presence of HCMV-infected target cells with or without autologous plasma as indicated. Dot plots show NK cells from one representative donor after 8 days in culture in indicated conditions. Numbers represent the percentage of conventional and FcRγ$^-$NK cells that are positive for the proliferation marker Ki-67. Dot graph depicts the change in frequencies of conventional (○) and FcRγ$^-$NK cells (●) compared to initial frequencies for individual donors after culturing for 11-13 d as indicated (*n*=6). Numbers represent the percentage of FcRγ$^-$NK and conventional NK cells. Dot graphs show the percentage of Ki-67$^+$ conventional NK cells and FcRγ$^-$NK cells from several donors in indicated culture conditions. Circles connected by a line designate the same donor sample. (**B**) PBMCs were first labeled with CFSE and cultured for 11 days. Shown are NK cells. Data show both FcRγ$^-$NK and conventional NK cells underwent cell division making nearly all cells CFSE dim, and were obtained from one representative donor. (**C**) After 6 days of culture as in (**B**), cells were labeled with CFSE, and further cultured for 5 days. Data show more FcRγ$^-$NK cells underwent more robust cell division than conventional NK cells, and were obtained from one representative donor.

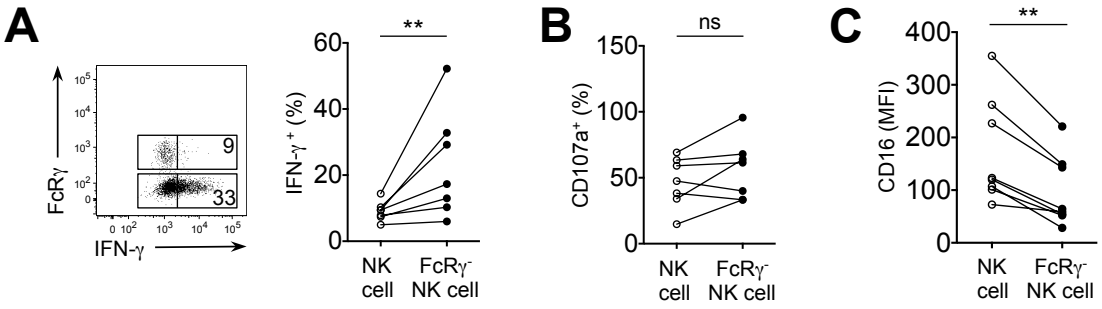

Figure S5 : Enhanced IFN-γ production response of expanded FcRγ$^-$NK cells without increased expression of CD16.

(**A**) Dot plot shows IFN-γ production by indicated subsets of NK cells from a representative donor after stimulation with immobilized anti-CD16 for 7 h. Numbers represent the percentage of NK cells that produced IFN-γ. Dot graphs show the percentage of conventional NK cells (NK) or FcRγ$^-$NK cells that produced IFN-γ from several donors. Circles connected by a line designate the same donor sample (*n*=7). (**B**) Cell surface expression of CD107a was determined following 7 h stimulation as in (**A**). Dot graph shows the percentage of conventional NK cells or FcRγ$^-$NK cells that displayed CD107a (*n*=7). (**C**) Dot graph shows MFI of surface expression of CD16 on conventional and FcRγ$^-$NK cells from expansion cultures. Circles connected by a line designate the same donor sample (*n*=8). ns, not significant; ** $P < 0.01$.

Supplemental Experimental Procedure

List of antibodies used in this study

The following antibodies were purchased from the indicated manufacturers and used for flow cytometry; BD Bioscience: anti-CD107a (H4A3), anti-CD16 (2.4G2), anti-CD3 (UCHT1), anti- IFN-γ (B27), anti-IKZF2 (22F6), anti-ILT2 (GHI/75), anti-KIR2DL2 (DX27), anti-ITGA6 (GoH3), anti- Ki-67 (B56) and anti-PLZF (R17-809); Beckman Coulter: anti-CD56 [N901 (HLDA6)]; Biolegend: anti-CD14 (HCD14), anti-CD19 (HIB19), anti-CD2 (RPA-2.10), anti-CD7 (6B7), anti-PECAM1 (WM59) and anti-SIGLEC7 (6-434); eBioscience: anti-FAS (DX2), anti-SYK (4D10.1) and anti-ZAP70 (1E7.2); Invitrogen: rabbit $F(ab)_2$ anti-human IgG; Life Tech: R-phycoerythrin goat anti-rabbit IgG (H+L); Milipore: anti-FcεRI subunit (FcRγ), anti-DAB2 (EP2297Y); Proteintech: anti-EAT-2 (SH2D1B), and R&D Systems: anti-NKG2C (134591) and anti-TIM-3 (84868).